PREVALENCE AND PREDICTORS OF METABOLICALLY HEALTHY OBESITY IN ADOLESCENTS

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Abstract

Background: A subset of individuals with obesity display a normal cardiometabolic profile, termed *metabolically healthy obes*ity (MHO).

Objectives: To examine the prevalence and predictors of MHO in adolescents.

Methods: Participants included 316 males and 316 females aged 12-19-years with a BMI ≥95th percentile from the 1999-2010 National Health and Nutrition Examination Surveys. First, MHO was defined as being free of type 2 diabetes, hypertension, and dyslipidemia and having <2 metabolic syndrome criteria. Second, MHO was defined as being free of all metabolic syndrome criteria, insulin resistance, and inflammation.

Results: The prevalence of MHO varied from 7-74% depending on the definition. Lower obesity and lower insulin-resistance predicted MHO in males and females (p<0.01). Associations between dietary components and MHO were weak and inconsistent. Physical activity and inflammation were not associated with MHO (p>0.05).

Conclusions: An emphasis on managing weight and insulin-resistance responses should be a central goal for adolescents with obesity.

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Abbreviations

CVD	Cardiovascular Disease
MHO	Metabolically Healthy Obesity
MUO	Metabolically Unhealthy Obesity
WC	Waist Circumference
BP	Blood Pressure
HDL-C	High-Density Lipoprotein Cholesterol
EOSS	Edmonton Obesity Staging System
LDL-C	Low-Density Lipoprotein Cholesterol
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
IDF	International Diabetes Federation
NCEP	National Cholesterol Education Program
CRP	C-Reactive Protein
MPA	Moderate Physical Activity
VPA	Vigorous Physical Activity
MVPA	Moderate-Vigorous Physical Activity
NHANES	National Health and Nutrition Examination Survey
BMI	Body Mass Index
PIR	Poverty Income Ratio
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
CI	Confident Interval
PR	Prevalent Risk

Chapter One: General Introduction

Adolescent obesity is associated poor dietary habits, insufficient physical activity and cardiometabolic health aberrations including hypertension, hyperglycemia and dyslipidemia[1]. Approximately 80% of obese adolescents become obese adults[2], and these obesity-related cardiometabolic health aberrations also track into adulthood[3] and increase the risk of incident type 2 diabetes, cardiovascular disease (CVD) and the risk of mortality[1]. However, despite the documented health risks associated with obesity, adolescent obesity is a heterogeneous condition wherein a subset does not present with these cardiometabolic risk factors[4], termed *metabolically healthy obesity* (MHO). There is a large discrepancy in the reported prevalence of MHO due to variations in the criteria used to define MHO, however a consistently lower prevalence is observed in males than females[4,5].

Some evidence suggests that adolescents with MHO may become healthy, non-obese adults or remain MHO as adults[6]. As most attempts to treat obesity have proven to have limited success[7], it may therefore be a more attainable goal for adolescents with obesity to achieve or maintain MHO as an early intervention. The determinants of MHO have been debated, but it has been suggested that differences in cardiometabolic function between adolescents with MHO and their peers, with metabolically unhealthy obesity (MUO) may be related to differences in diet or physical activity habits[8], or variability in the level of obesity[8], obesity-related sub-clinical

inflammation[9] or insulin-resistance responses[9]. As diet and physical activity habits also tend to track into adulthood[10], diet and physical activity may be important modifiable factors for maintaining metabolic health in obesity.

To our knowledge, no study to date has used a large nationally representative sample to determine which of these factors is most important in predicting MHO in adolescents. The objective of this study was to investigate the prevalence of adolescents with MHO and the independent relationships between obesity, diet, physical activity, insulin-resistance and inflammation with MHO in male and female adolescents.

Chapter Two: Review of the Literature

Introduction

The prevalence of obesity in adolescents has tripled in the past three decades, reaching 21% in American adolescents[11] and 10% in Canadian adolescents[12]. Obesity is associated with an array of health conditions including but not limited to type II diabetes, cardiovascular disease, certain types of cancer, gout, osteoarthritis, reproductive disorders, depression, chronic obstructive pulmonary disease, non-alcoholic fatty liver disease, and back pain[13]. These obesity-related health conditions pose a large burden on the health care system, costing Canada approximately 11 billion[13] and the US approximately 13 billion[14] dollars annually in health care services. While most of these obesity-related health conditions do not become apparent until adulthood, certain comorbidities associated with obesity once thought to only be applicable to adults, are now increasing at an alarming rate in adolescents[15]. For instance, most adolescents with obesity have at least one additional risk factor for type II diabetes and CVD[16], such as hypertension, dyslipidemia, hyperglycemia, insulin-resistance and inflammation, which are termed cardiometabolic risk factors[1]. Unfortunately, these metabolic aberrations tend to track into adulthood[3], and prolonged exposure may result in irreversible damage[1].

Metabolic Health

The metabolic syndrome is a condition characterized by the clustering of at least three of the following cardiometabolic risk factors: elevated waist circumference (WC), blood pressure (BP), glucose, triglycerides and low high-density lipoprotein cholesterol (HDL-C)[1]. Early and continued development of these cardiometabolic risk factors, clustered or in isolation, has been demonstrated to increase and accelerate the risk for developing type II diabetes and CVD later in life[1]. With the increasing prevalence of the metabolic syndrome in youth[15], it has been proposed for first time in modern history, that youth may have a shorter life expectancy than their parents[15].

Although obesity is associated with an increased risk of developing health complications, variability exists in the quantity and severity of comorbidities experienced by individuals with obesity[17]. Some individuals with obesity are free of comorbidities, while others display an array of health complications[17]. Consequently, numerous classification systems have been developed to diagnose the clustering and/or severity of health risks associated with obesity. For instance, a recently proposed obesity classification system, known as the Edmonton Obesity Staging System (EOSS)[18], classifies adults with obesity according to a 5-point ordinal scale based on psychological, cardiometabolic, and functional status[18]. On the lower end of the scale, the EOSS stage 0 represents adults with obesity that are free of obesity-related health complications and, on

the other end of the scale, stage 4 represents end-stage conditions requiring palliative care[18]. Other methods of classifying the health risks associated with obesity include the classification of adults with obesity according to the presence of the metabolic syndrome[17], metabolic syndrome criteria[17], and/or other cardiometabolic risk factors such as inflammation[19], insulin-resistance[19], elevated low density lipoprotein cholesterol (LDL-C)[20], and/or elevated total cholesterol[20]. These classification systems often differentiate individuals with obesity having limited comorbidities, termed metabolically healthy obesity (MHO), from individuals displaying an array of cardiometabolic health conditions, termed metabolically unhealthy obesity (MUO).

Although fewer classification systems have been proposed to diagnose the health risks associated with obesity in adolescents, most differentiate MHO from MUO in adolescence using markers of insulin-sensitivity or metabolic syndrome criteria. Most commonly, insulin-sensitivity is assessed using the homeostatic model assessment of insulin-resistance (HOMA-IR)[8,9], whereas studies classifying adolescents using the absence of the metabolic syndrome often use a variety of criteria, such as pediatric IDF criteria[4,21], modified NCEP III criteria[22] or age-specific criteria[5] for the metabolic syndrome, or combinations of these approaches to define MHO. Most MHO definitions have unique cut-points for the levels of BP, glucose, triglycerides, and HDL-C or HOMA-IR considered as elevated as metabolic syndrome criteria cut-offs themselves and what the presence of metabolic syndrome in adolescences means are debated.

These differences in defining 'metabolically healthy' make it difficult to compare adolescents with MHO from one study to another. These discrepancies may account for some of the variance in the reported prevalence of MHO, which ranges from 16-68%[4,5,8,9,21,22].

Although most report that more than half of adolescents with obesity have MHO[4,9,21,22], adolescents with isolated type 2 diabetes, dyslipidemia or hypertension are often not excluded from the group with MHO unless these adolescents experience clustering of these conditions with other cardiometabolic risk factors. However, it is questionable whether an individual with isolated type 2 diabetes can in fact be called 'metabolically healthy'. Therefore, in the present study we will investigate the prevalence and predictors of MHO using both a traditional and a more conservative definition as well as differences between these two definitions.

Etiology

The etiology of cardiometabolic aberrations in individuals with MUO is multifactorial, however, three main culprits may be responsible for this cardiometabolic dysfunction: obesity, insulin-resistance, and low-grade inflammation[1]. Lifestyle factors such as diet and physical activity have also been demonstrated to influence obesity, insulin-resistance, low-grade inflammation, and MUO[8,23]. The following section will provide a brief overview of the pathogenesis relating obesity, insulin resistance, low-grade inflammation, diet, and physical activity to the development of MUO.

Obesity

Adipose tissue is a specialized tissue that stores energy in the form of triacylglycerols and also acts as an endocrine and paracrine organ releasing adipokines that may participate in the pathogenesis of obesity related comorbidities[1]. During a period of positive energy balance, adipocytes expand, becoming larger in size (hypertrophy) and can trigger the generation of new adipocytes (adipogenesis) to increase the storage capacity for the excess triacylglycerol[24]. However, in some individuals, adipogenesis is impaired and is unable to match the need, and thus storage of triacylglycerols is achieved through hypertrophy in larger adipocytes, or in deposition of lipids in nonadipose tissues (ectopic fat), resulting in lipotoxicity[25]. The size of the adipocytes is positively associated with the quantity of adipokines released, thus, individuals with obesity with larger adipocytes have greater adipokine secretion than individuals with a normal weight or obesity with smaller adipocytes[26]. Adipocyte hypertrophy and adipokine hyper-secretion often lead to endoplasmic recticulum stress, apoptosis, macrophage infiltration, chronic low-grade inflammation, insulin resistance and the release of triglyceride-derived free fatty acids, all of which are related to MUO[25]. Consequently, the severity of obesity, which is associated with the size of adipocytes, is positively associated with cardiometabolic dysfunction[1] and inversely associated with MHO[8].

Not only is the severity of obesity associated with MUO, but also the distribution of

adipose tissue[24]. Two main types of adipose tissue exist: subcutaneous adipose tissue and visceral adipose tissue. Subcutaneous adipose tissue comprises the adipose tissue depots found between the skin and the aponeuroses and fasciae of the muscles[24] and visceral adipose tissue comprises the adipose depots found in the intrathoracic and intra-abdominopelvic regions, including ectopic fat in the liver, pancreas, etc[24]. It has been hypothesized that excess visceral adiposity may be caused by the inability of subcutaneous adipose tissue to expand through adipogenesis during a period of positive energy balance[27]. Differences are observed in the methods by which these adipose tissues are vascularized, innervated and to which blood flows to and from these areas[24]. For instance, visceral adipose tissue is drained by the portal vein leading to the liver[24]. Free fatty acids released from visceral adipose tissue thereby accumulate in the liver where they stimulate the production of very low-density lipoprotein triglycerides leading to insulin-resistance, dyslipidemia, and elevated blood pressure[24]. The strongest correlate of visceral fat is waist circumference, and thus abdominal obesity is consistently associated with a lower likelihood of MHO than general obesity in adults[19] and youth[8].

Insulin Resistance and Low-grade Inflammation

Insulin resistance and low-grade inflammation are also commonly proposed culprits of cardiometabolic dysfunction that appear to be inter-related[1]. Insulin resistance is a pathophysiological condition characterized by the inability of normal insulin concentrations to

produce a normal insulin response in peripheral tissues such as the muscle, liver, pancreas and adipose tissues[26]. Pancreatic beta cells must therefore increase the secretion of insulin (hyperinsulinemia) to overcome hyperglycemia[26]. Insulin resistance results in increased circulation of free fatty acids and hyperglycemia, and is thought to be the central metabolic abnormality in CVD and type 2 diabetes[26].

Chronic low-grade inflammation is characterized by the accumulation of lymphocytes, macrophages and plasma cells in tissues[28]. Low-grade inflammation induces the cardiometabolic aberrations observed in individuals with MUO via several mechanisms. Inflammatory cytokines induce adipocyte apoptosis and increase insulin resistance by inhibiting the insulin receptor substrate 1 signaling pathway[26]. Inflammatory cytokines can also suppress lipoprotein lipase activity and increase hepatic synthesis of free fatty acids, resulting in dyslipidemia[29]. Inflammatory cytokines thus mediate several inter-related alterations in cardiometabolic pathways including free fatty acid metabolism, insulin sensitivity, oxidative stress, and the metabolic aberrations observed in MUO[1]. Therefore, both HOMA-IR and C-reactive protein (CRP), commonly used measures of insulin resistance and inflammation, respectively, have been demonstrated to be inversely associated with MHO in adolescents with obesity[21]. However, which of these factors is most central to metabolic function is still unclear.

It is important to note that not all individuals with obesity experience insulin resistance

and/or low-grade inflammation and some individuals with a normal weight experience these cardiometabolic aberrations[1]. Evidence exists to suggest that alternative factors including but not limited to dietary factors[8,23], smoking[28], gut microbiota[28], or inadequate physical activity[23,30] may be the causal factors driving the insulin resistance and/or low-grade inflammation, and subsequently resulting in the obesity of the individual as well as the cardiometabolic aberrations observed in individuals with MUO[31]. For example, studies in both mice and rats have demonstrated that inflammation may result from these alternative factors before the appearance of obesity and disabling these inflammatory pathways has prevented the development of obesity under similar obesity-inducing conditions[31]. Similarly, longitudinal studies in humans have demonstrated that accelerated weight gain is preceded by changes in inflammatory cytokines[29]. However, temporal and causal relationships of obesity, insulin resistance and inflammation with MUO remain unclear[29].

As the hypotheses suggesting that obesity, insulin-resistance or low-grade inflammation result in the metabolic derangements of MUO are not mutually exclusive, all three are suggested to play a role in the pathogenesis of cardiometabolic risk factor clustering[1]. Therefore, it has been proposed that the differences in metabolic function between the MUO and MHO phenotypes may be related to the individual variability in obesity, inflammation, and/or insulin-resistance[21].

Diet

Energy restriction, as well as several alterations in macronutrient composition have been demonstrated to improve body composition and overall cardiometabolic health[32]. Even without caloric restriction, decreasing dietary carbohydrate intake, or replacing high glycemic index carbohydrates with fiber-dense carbohydrates, has been suggested to decrease plasma triglycerides, increase HDL-C, reduce plasma glucose concentrations, and lower BP[32,33]. Similarly, decreasing total fat intake, especially saturated fat intake and cholesterol are alternative methods of improving cardiometabolic health[33]. Further, replacing saturated fat with polyunsaturated and monounsaturated fats have been demonstrated to improve components of cardiometabolic health, particularly insulin resistance and inflammation[32]. Therefore, maintaining a balance between carbohydrate and fat consumption, while limiting saturated fat, simple carbohydrate, and cholesterol intake may help maintain or improve cardiometabolic health. The current recommendations suggest limiting total fat intake to approximately 30% of daily caloric intake and saturated fat to 7-10% of daily caloric intake, while limiting cholesterol intake to 300mg per day[34]. At least 14 g/1000 kcal should be derived from fiber and carbohydrates should comprise approximately 50-55% of daily caloric intake and proteins approximately 15-20% of daily caloric intake[34].

Studies also suggest that diet influences cardiometabolic health in youth[35], however

research in youth is less abundant. The few studies that have investigated these associations have suggested that lower fiber[36], polyunsaturated fat intake[37] and protein[38] and higher carbohydrate[39], saturated fat[40], and total fat[39] are associated with poorer cardiometabolic health. Further, only one study has investigated the association between dietary components specifically with MHO in adolescents and demonstrated that total fat intake is inversely associated with MHO, independent of waist circumference and physical activity[8]. Since limited research is available on diet and MHO in the adolescent population, further studies are required to clarify these associations. In the present study, we will examine the associations between total fat, saturated fat, polyunsaturated fat, monounsaturated fat, carbohydrates, protein, fiber and cholesterol with MHO in adolescents with obesity.

Physical Activity

Physical activity has been demonstrated to increase physical fitness, decrease adiposity and enhance overall cardiometabolic health[41]. The greatest health benefits of physical activity are observed when transitioning from a state of inactivity to being active[42]. Most of these health benefits can be acquired through moderate physical activity (MPA), although additional benefits are provided from vigorous physical activity (VPA)[41]. The associations between physical activity and cardiometabolic health occur independent of obesity, however, they are weakened when researchers use self-reported measures of physical activity[41].

The health benefits of physical activity are also observed in adolescents with obesity[43], however, the association of physical activity specifically with MHO in adolescents with obesity has not yet been firmly established, as only two studies have investigated this association. The first study used nationally-representative and self-reported data to examine MPA, VPA, moderate-vigorous physical activity (MVPA), muscle strengthening activities, active transportation, and physical activity as a function of metabolic equivalent of task (in minutes per week). Despite the large number of analyses, none of the physical activity variables were significantly associated with MHO[4]. Contrarily, data from a clinical pediatric weight management data, report a positive association between MVPA (in minutes per day) and MHO independent of obesity and diet, also using self-reported data[8]. It is unclear why conflicting results were obtained from these studies, but due to the lack of studies examining this area, more research is needed. The present study will further investigate the association between physical activity and MHO in attempt to add clarification to the limited and inconclusive literature. As evidence exists to suggest that the association between physical activity and cardiometabolic health in youth is weaker in females[41], the present study will investigate the association between physical activity and MHO in adolescents with obesity by sex.

Summary of literature

Unfortunately, few studies have investigated the prevalence and predictors of MHO in

adolescents with obesity in comparison to adult literature and most have included overweight adolescents in their analyses, portraying an inflated prevalence of MHO. Furthermore, no study to date has examined the independent associations between obesity, diet, physical activity, and laboratory measures (insulin-resistance and inflammation) in relation to MHO using a nationally-representative sample of adolescents with obesity. Therefore the objectives of the present study are to investigate:

- The prevalence of MHO in male and female adolescents with obesity using two definitions of MHO.
- The association between obesity, diet, physical activity and laboratory measures with MHO in adolescents with obesity, by sex.
- The independent associations between obesity, diet, physical activity and laboratory measures with MHO in adolescents with obesity, by sex.

Chapter Three: Methods

Data Source and Sampling

Data from the 1999-2010 years of the National Health and Nutrition Examination Survey (NHANES), an ongoing, cross-sectional study was analyzed. The NHANES is conducted by the National Center for Health Statistics for the Centers for Disease Control and Prevention and is composed of interview and physical examination components to assess the health and nutritional status of the U.S. population. The NHANES uses a complex four-stage probability sampling method to obtain a nationally representative sample of the noninstitutionalized U.S. population. Informed consent was obtained from 18 and 19 year old participants, and parental consent and participant assent was obtained for participants under the age of 18 years. All laws regarding human ethics were followed and the protocol was approved by the National Center for Health Statistics Ethics Review Board. Details of the survey methods are described elsewhere[44].

Subjects

The present study included NHANES participants ages 12 to 19 years with an age- and sex-specific BMI $\geq 95^{th}$ percentile[45] (n= 2370). Participants who were *(i)* pregnant (n = 47), *(ii)* taking insulin (n=6), *(iii)* using tobacco or nicotine products within the past five days (n = 272), *(iv)* fasted for less than three hours[46,47] (n = 438) and (v) having CRP concentrations greater than 10mg/L[48] (n = 1) were excluded. Individuals with variable outliers, implausible values, or

missing values for either WC (n = 63), tobacco use within the past five days (n = 178), MPA (n = 93), VPA (n = 81), energy intake (n = 84), total fat intake (n = 88), saturated fat intake (n = 90), monounsaturated fat intake (n = 83), polyunsaturated fat intake (n = 93), carbohydrate intake (n = 83), protein intake (n = 85), fiber intake (n = 86), cholesterol intake (n = 97), fasting triglycerides (n = 1383), fasting glucose (n = 1376), fasting insulin (n = 1385), fasting HDL-C (n = 228), CRP (n = 220), poverty income ratio (PIR; n = 180), systolic blood pressure (SBP; n = 90) and diastolic blood pressure (DBP; n = 110) were also excluded from the analysis. The final sample included 316 male and 316 female adolescents with obesity.

Demographic variables

Information on age, gender, family size and income was obtained via home interviews. Demographic data were reported by a proxy for participants under the age of 16 years and were self-reported for participants aged 16 to 19 years. Family size and income were used to compute PIR, a measure of socioeconomic status calculated as family income divided by the Department of Health and Human Services' poverty guidelines based on family size, year and state[49]. Values for PIR are provided on a continuous scale from "0" to "5", with a value of "1" representing the poverty line.

Anthropometric Measures

WC was measured without clothing, to the nearest 0.1cm, at the midpoint between the

bottom of the rib and the top of the iliac crest during minimal respiration. Weight was assessed to the nearest 0.1kg with a Toledo digital scale (Mettler-Toledo Inc, Columbus, OH) while participants wore underwear, disposable paper gowns and foam slippers. Normal WC was defined as having a WC $\leq 90^{th}$ percentile for age and sex[50] or the adult cut-off, if lower[51]. Height was measured to the nearest 0.1cm using a fixed stadiometer. BMI percentiles were calculated from weight and height measurements using the Centers for Disease Control and Prevention age- and sex- specific growth charts[45].

Diet

Data on diet was obtained using a 24-hr diet recall method. Participants were asked to list the volume of all food and beverages consumed from midnight to midnight the day prior to arriving at the mobile examination center. Measuring tools and food models were provided to aid participants with quantifying proportions. Trained dietary interviewers recorded this information using the USDA automated pass method[52]. During the years 2003-2010, the average of the two 24-hour diet recalls was used. The dietary variables assessed in the present study include: energy intake (kcal), total fat (g), saturated fat (g), monounsaturated fat (g), polyunsaturated fat (g), carbohydrates (g), protein (g), fiber (g) and cholesterol (mg). Further details on NHANES diet-recall procedures can be found elsewhere[53]

Recreational Physical Activity

Participants completed a questionnaire on physical activity, derived from the World Health Organization's Global Physical Activity Questionnaire[54], in the mobile examination center for 12-15 year old participants and in the participant's homes for 16-19 year old participants. Participants were asked to disclose information on frequency and duration (in minutes) of all recreational MPA and VPA they had engaged in for at least 10 minutes continuously, "in the past 30 days" for NHANES 1999-2006 and "in a typical week" for NHANES 2007-2010. Recreational MVPA was calculated as the sum of recreational MPA and VPA in minutes per week. Details on the NHANES physical activity questionnaire have been reported previously[53].

Laboratory Measures

SBP and DBP are an average of two or three readings from a manual mercury sphygmomanometer in the mobile examination center following the protocol of the American Heart Association. The use of hypertension medication was assessed via questionnaire. Blood samples were drawn from the participants arm by a certified phlebotomist in the mobile examination center, processed, stored and shipped to various laboratories for analysis. Fasting plasma glucose was measured using hexokinase methods. Plasma insulin was measured using the radioimmunoassay double-antibody batch method in 1999-2002, the two-site

immunoenzymatic assay in 2003-2005, and the human insulin immunoassay method (ELISA) in 2005-2010. HOMA-IR[55] was calculated as: fasting insulin (mU/L) x fasting plasma glucose (mmol/L)/ 22.5. Blood CRP was quantified with a Behring Nephelometer. Serum triglycerides were assessed enzymatically using a series of coupled reactions in 1999-2006, and a two-reagent end-point reaction in 2007-2010. HDL-C levels were assessed using the Heparin-Mn precipitation method in 1999-2001 and the direct HDL method in 2003-2010. Triglyceride and HDL-C levels were measured using a Hitachi 704 Analyzer in 1999-2004, Roche Hitachi 717 in 2005, Roche Hitachi 717 and 912 in 2006, and a Roche Modular P chemistry analyzer in 2007-2010. Details of all NHANES laboratory protocol and procedures are available elsewhere[53].

Definition of MHO

Two methods were used to define MHO. The primary definition excluded adolescents with: *(i)* clinically diagnosable levels of fasting plasma triglyceride, glucose, HDL-C, and BP and *(ii)* two or more pre-clinical metabolic syndrome criteria (**Table 1**). The secondary definition of MHO was more stringent as it excluded adolescents with: *(i)* any pre-clinical metabolic syndrome criteria *(ii)* insulin resistance or *(iii)* inflammation (**Table 1**).

Statistical Analysis

All statistical analyses were performed using SAS software version 9.3 (SAS Institute, Cary, NC) with a significance level of $p<0.05$. Analyses were stratified by sex. Due to the

complex sampling design of NHANES, sample weights were applied as recommended by the National Center for Health Statistics[56]. Descriptive data are presented as means±standard errors and differences between MHO and MUO groups were determined using independent samples t-tests. In multivariable analyses, predictors were standardized using standard error to allow for comparisons between variables. Logistic regressions adjusted for age and socioeconomic status were performed on the standard error of each independent variable using Proc Surveylogistic. Due to the high prevalence of MHO in the study sample, Odds Ratios and 95% Confidence Intervals (CI) were then transformed into Prevalent Risk (PR) and 95% CI.[57]. The most significant predictor of MHO from each category (obesity, diet, physical activity, laboratory measure-primary MHO definition only) was then entered into a mutually-adjusted model with further adjustment for age and socioeconomic status.

Chapter Four: Results

Participant Characteristics

Figure 1A displays the proportion of adolescents with each cardiometabolic risk factor and **Figure 1B** displays the number of pre-clinical metabolic syndrome risk factors (triglycerides, glucose, HDL-C and BP) in the study sample. The percentage of adolescents free of any of the four pre-clinical metabolic syndrome risk factors was 27% in males and 45% in females, and the proportion of adolescents with 0 or 1 pre-clinical metabolic syndrome risk factors was 55% in males and 79% in females (**Figure 1B**). After excluding individuals with clinically diagnosable levels of metabolic syndrome risk factors, the prevalence of MHO was 42% in males and 74% in females (Primary Definition). Characteristics of the study sample by sex and metabolic health status are presented in **Table 2**. Using the primary definition of MHO (<2 pre-clinical metabolic syndrome risk factors and no clinically diagnosable levels of metabolic syndrome risk factors), MHO males were younger, of a lower socioeconomic status, less obese, ate more total fat, saturated fat, monounsaturated fat, protein, fiber and cholesterol and were less insulin-resistant than MUO males. MHO and MUO females did not differ in age, socioeconomic status, obesity, diet, or physical activity behaviours, however, they were less insulin-resistant than MUO females. According to the secondary definition of MHO (no metabolic syndrome criteria, insulin-resistance and inflammation), the prevalence of MHO was

only 7% in males and 12% in females. MHO males were of a higher socioeconomic status, less obese, reported a lower fiber intake, and participated in more MPA and VPA than MHO males. MHO females had a higher socioeconomic status, lower WC and participated in less VPA than MUO females.

Predictors of MHO

Using the primary definition of MHO, in males, BMI percentile, WC and carbohydrate intake were negatively associated with MHO and protein intake and cholesterol intake were positively associated with MHO (**Table 3**). Physical activity did not predict MHO. Lower insulin-resistance but not inflammation was associated with MHO. In females, obesity was not associated with MHO and, of the dietary components, only a higher polyunsaturated fat intake predicted MHO. Physical activity was not associated with MHO. Lower insulin-resistance, but not inflammation was associated with MHO. According to the secondary definition of MHO, in males, BMI percentile and WC were negatively associated with MHO (**Table 3**). Unexpectedly, of the dietary components, only saturated fat intake was positively associated with MHO. Physical activity was not associated with MHO. In females, lower WC and higher monounsaturated fat intake were associated with MHO. Physical activity was not associated with MHO.

Independent Predictors of MHO

To determine the independent associations between obesity, diet, physical activity, and laboratory measures with MHO, the most significant obesity, diet, physical activity and laboratory correlates (Definition 1 only) of MHO were entered into a mutually adjusted model with further adjustment for age and socioeconomic status (**Table 4**). In males, the model for the primary definition of MHO included WC, carbohydrate intake, VPA and insulin-resistance, of which only WC and insulin-resistance remained significantly associated with MHO (**Table 4**). In females, the mutually adjusted model for the primary definition of MHO included WC, polyunsaturated fat, MVPA and insulin-resistance, of which higher polyunsaturated fat intake and lower insulin-resistance remained independently associated with MHO. The model for the secondary definition of MHO in males included WC, saturated fat and MVPA, of which WC remained negatively associated with MHO. The model predicting the secondary definition of MHO in females included WC, monounsaturated fat and VPA, of which only WC remained associated with MHO.

Chapter Five: Discussion

The present study investigated the prevalence and predictors of MHO in a nationally representative sample of adolescents. We demonstrated that only 7% of males and 12% of females with obesity could be classified as MHO using the most stringent definition, while 42% of males and 74% females were classified as MHO using a more commonly used definition. Lower WC and insulin-resistance were the most consistent predictors of MHO in both males and females. No individual dietary component was consistently related to MHO and physical activity did not predict MHO. Our data suggest that adolescents with obesity should strive to achieve or maintain lower WC and insulin-resistance.

Previous literature reporting the prevalence of MHO has used several definitions of the metabolic syndrome including the absence of insulin-resistance[9] age-specific criteria[5], pediatric IDF criteria[4,21,5,58], modified NCEP III criteria[22] or combinations of these approaches[8,9] to define MHO. Depending on the definition and the number of criteria used to define MHO, there can be large variations in the prevalence of MHO ranging from 16-68% in previous literature[4,5,8,9,21,22,58] and 7-79% in our study. This variability emphasizes the need to harmonize the definition of MHO in adolescents. We suggest that our primary definition may be reasonable criteria as it considers not only the number of metabolic conditions, but also the severity, to determine metabolic health status. This means that our MHO adolescents do not have clinically

diagnosable comorbidities or clustering of pre-clinical risk factors: both of which are clear markers of ill health.

The etiology of cardiometabolic health has been debated with some arguing that insulin resistance is the central feature, while others suggest that obesity is the primary factor[29]. Obesity is one of the most consistent predictors of MHO in adolescents in the current study and past literature[5,8]. Insulin resistance is not examined as frequently, but is also identified as an important predictor of MHO[21,59]. Our study reports that both abdominal obesity and insulin resistance are predictors of MHO in obese adolescents, independent of lifestyle factors. Insulin-resistance, however was observed to be more closely associated with MHO than obesity, especially in females. Obesity, insulin-sensitivity and metabolic health are highly inter-related and co-exist in adolescents with obesity. Within our MHO youth (Definition 1), we observe that although 31% were insulin sensitive, only 7% had a normal WC, and only 4% had both insulin sensitivity and a normal WC (data not shown). While it is debated whether insulin-resistance precedes obesity or obesity precedes insulin-resistance, both obesity and insulin-resistance have been proposed to be the responsible for cardiometabolic dysfunction[29], and are influenced by lifestyle factors.

In comparison to the literature on obesity and insulin-resistance, the associations between dietary components and cardiometabolic health in youth are weak and inconsistent. For instance,

lower fiber[36], polyunsaturated fat intake[37] and protein[38] and higher carbohydrate[39], saturated fat[40], and total fat[39] have all been inconsistently associated with poorer cardiometabolic health in youth. However, only one previous study has investigated the relationship between dietary components and MHO in youth and reported a negative association between dietary fat intake with MHO[8]. The present study presents conflicting results, as only a higher polyunsaturated fat intake is independently related with MHO in females, whereas diet does not independently predict MHO in males. These inconsistent findings may highlight the complexity of how diet relates to health, as there may be several dietary approaches by which to attain a lower body weight or improved insulin sensitivity that are not fully captured by our statistical analyses.

Studies on physical activity have been more consistent in demonstrating positive effects on cardiometabolic health in youth[43]. These effects are mainly related to improvements in adiposity and cardiorespiratory fitness, although independent effects are also observed[43]. Indeed, physical activity is associated with acute improvements in several facets of health[60] The relationship between physical activity and MHO in youth is less clear as only three studies have investigated this relationship. One of these studies reported a positive association[8] between physical activity and MHO, whereas the study, by Camhi et al.[4] as well as the present study reported no association. The absence of an association between physical activity and MHO in the present study may be explained by the high prevalence of adolescents participating in

physical activity in our sample (87%; data not shown) as most health benefits are obtained when transitioning from a state of inactivity to being active[61]. Alternatively, factors such as musculoskeletal or cardiorespiratory fitness or sedentary behavior were not available but may provide added insight as to why some obese youth develop metabolic aberrations versus those who do not.

This study has limitations to consider. First, several factors that have been demonstrated to influence the MHO status of adolescents were not accounted for, such as visceral fat[62], neck circumference[9], uric acid concentration[21], adiponectin[63] and hepatic steatosis[5]. Second, data on diet and physical activity is subject to self-reporting biases, for example under-reporting is especially pronounced in obese and adolescent populations[64]. However, this should not affect the integrity of our results, unless MHO and MUO adolescents reported behaviours differently. Also, data on diet for the years 1999-2002 was based solely on one 24-hr diet recall and in the years 2003-2010, data was an average of only two 24-hr diet recalls. This may have been insufficient to capture the regular dietary habits of these adolescents and may explain the unexpected relationships observed between dietary components with MHO in males. Further, MUO adolescents may have been actively engaging in healthier behaviours to reduce obesity-related comorbidities. Finally, this study is cross-sectional in design, thus causality cannot be inferred from the associations observed.

In conclusion, this study demonstrated that 7 to 74% of youth with obesity are metabolically healthy, depending on the definition used. The likelihood of being MHO significantly decreases with higher levels of obesity and insulin-resistance. Conversely, diet, physical activity and inflammation were not as consistent in distinguishing MHO from MUO adolescents. These findings highlight the need for effective strategies of managing obesity and insulin-resistance to achieve or maintain metabolic health in adolescents with obesity.

Chapter Six: Acknowledgements

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Chapter Seven: General Discussion

Many changes in society may have contributed to today's rise in obesity, including but not limited to the adoption of sedentary lifestyles and the abundance of readily available foods with a high caloric content[65]. The obesity pandemic is not only apparent in developed countries but is also rapidly emerging in developing countries, even amongst children and adolescents[65]. In North America, the prevalence of obesity in adolescents has tripled in the past three decades and the prevalence of abdominal[13] and severe obesity[66] has increased at an even faster rate than general obesity. This is alarming, as we have demonstrated that abdominal obesity is one of the most consistent predictors of cardiometabolic health in adolescents, even in our study with a narrow obese range. Further, previous studies demonstrate that obesity tracks well into adulthood[67], therefore the future metabolic health of the abdominally obese adolescents is unclear. Insulin-resistance was also associated with MHO and is strongly associated with abdominal obesity[24]. Thus, the prevention, management and treatment of obesity, particularly abdominal obesity, may be a solution to managing overall cardiometabolic health in adolescents. However, obesity management at the population level has proven to be extremely challenging, with not one country to date having succeeded in reducing its obesity rates[68]. Fortunately, many policy makers have taken interest in addressing the issue of obesity and a multitude of research is currently published on the topic. In Canada alone, the Canadian Obesity Network comprises

over 10,000 members including health care providers, policy makers, researchers and policy stake holders that are working together to prevent, manage, and treat obesity as well as obesity-related health complications[69].

Still, the question of how to address the issue of obesity in adolescents remains unanswered. As only 7-9% of adolescents with obesity are free of comorbidities, promoting obesity management and treatment amongst all adolescents with obesity, regardless of the presence of comorbidities, may be a simple yet functional method of improving cardiometabolic health in adolescents at a population level. In fact, promoting weight loss even for adolescents with MHO may be beneficial for numerous reasons: *i)* obesity-related comorbidities may still develop with age[70], *ii)* other psychological or health issues may be present despite the absence of cardiometabolic aberrations, *iii)* the incidence of mortality is increased in all individuals with obesity, regardless of metabolic health status[17] and *iv)* beneficial effects on cardiometabolic health are observed with a only modest decrease in body weight percentage, even in individuals with MHO[71]. However, prescribing weight loss and/or obesity management to all adolescents, including MHO adolescents may lead to frustration as a low rate of weight loss maintenance exists in the obese population[72] and as a result, attempts to lose weight often lead to weight cycling which is associated with cardiometabolic dysfunction[73]. Taking these factors into consideration, it is unclear whether weight loss or weight maintenance should be promoted in all

adolescents with obesity, including adolescents with MHO. In adults, the EOSS recommends weight loss for MUO individuals and the prevention of further weight gain for MHO individuals[18]. A scale has not yet been fully developed for pediatrics, however, preliminary work has been completed, suggesting that pediatric recommendations for weight management according to health status are likely forthcoming[74].

Although we have demonstrated that lower obesity and insulin-resistance are associated with a healthier cardiometabolic profile, physical activity was not associated with MHO and associations between diet and MHO were inconsistent across definitions of MHO. Therefore, it is possible that methods by which a healthier cardiometabolic profile are maintained through diet and physical activity may vary from individual to individual. For instance, diet and physical activity behaviours that influence the cardiometabolic health of one adolescent with obesity may not have the same influence on another adolescent with obesity. Alternatively, there may be multiple combinations of diet and physical activity behaviours that are associated with lower obesity and a healthier cardiometabolic profile, therefore not one single dietary or physical activity component alone would consistently be related to MHO.

In conclusion, the present study has demonstrated that the prevalence of MHO varies considerably according to the criteria used to define MHO. Therefore, future studies may benefit from harmonizing the definition of MHO. We propose a definition of MHO that excludes

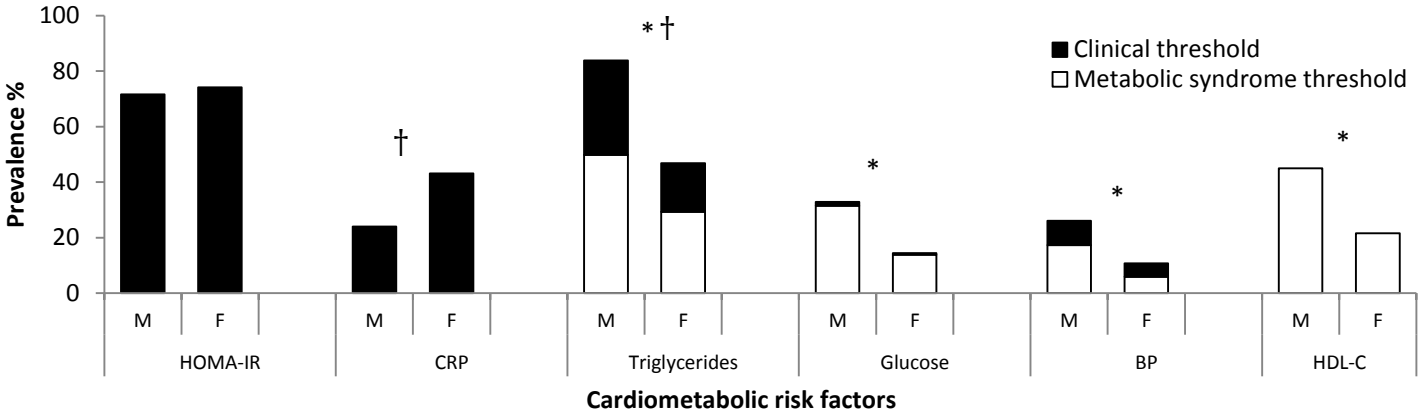
adolescents with clinically elevated comorbidities and clustering of pre-clinical cardiometabolic risk factors, both of which are markers of ill health. The present study has also investigated the association of obesity, diet, physical activity and laboratory measures with MHO during adolescence, a time in life where health behaviours and cardiometabolic risk factors track into adulthood. We have demonstrated that both lower obesity and lower insulin resistance are consistently associated with MHO. Further research is required to investigate successful methods of attaining and maintaining lower obesity and insulin resistance at a population level.

Table 1: Clinical and metabolic syndrome thresholds of cardiometabolic risk factors.

Risk Factor	Pre-clinical Metabolic Syndrome Threshold	Clinical Threshold
Triglycerides	≥ 1.24 mmol/L[75]	≥ 1.47 mmol/L[34]
Glucose	≥ 5.55 mmol/L[34]	≥ 7.0 mmol/L[76]
HDL-C	≤ 1.04 mmol/L[34]	< 1.04 mmol/L[34]
BP	SBP or DBP ≥90th percentile for sex, age and height for 12-17 year old youth[34] and ≥130/85 mmHg for 18 and 19 year old youth[75]	current use of hypertension medication or SBP or DBP ≥95th percentile for sex, age and height[34] for 12-17 year old youth and SBP or DBP≥140/90 mmHg for 18 and 19 year old youth[34]
CRP		≥ 3 mg/L[48]
HOMA-IR		≥ 3.16[77]

Abbreviations: HDL-C: High-Density Lipoprotein Cholesterol, BP: Blood Pressures, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, CRP: C-Reactive Protein, HOMA-IR: Homeostatic Model Assessment of Insulin Resistance.

1A



1B

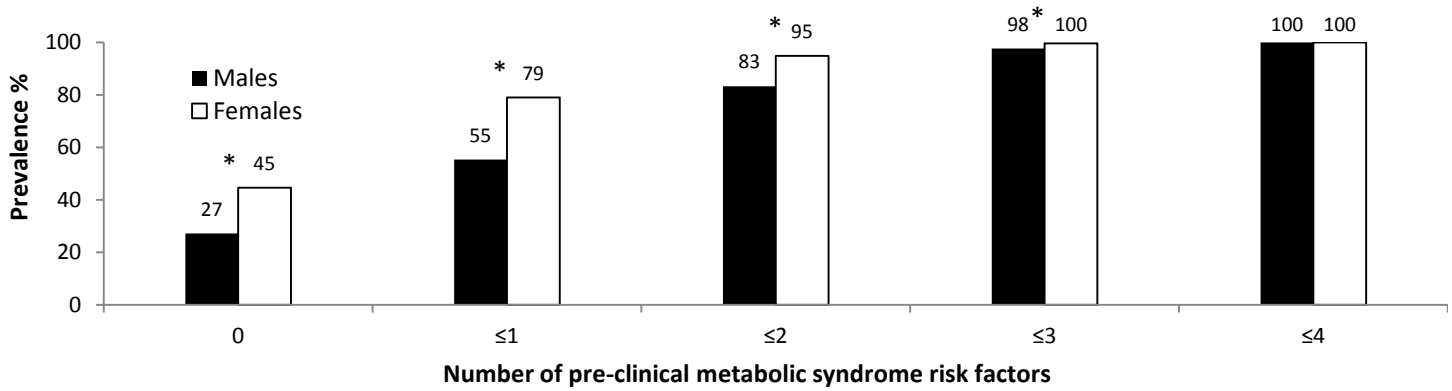


Figure 1A&B: Prevalence of cardiometabolic risk factors in male and female adolescents.

Clinical thresholds: HOMA-IR≥3.16, CRP≥3.0 mg/L, triglycerides≥1.47mmol/L, glucose≥7.0mmol/L and BP (currently taking medication for hypertension or SBP or DBP ≥95th percentile for sex, age and height for youth under 18, and ≥140/90 mmHg for 18 and 19 year old youth). Metabolic syndrome thresholds: triglycerides≥1.24 mmol/L, glucose≥5.55 mmol/L, BP (SBP or DBP ≥90th percentile for sex, age and height for youth under 18, and ≥130/85 mmHg for 18 and 19 year old youth), HDL-C≤1.04 mmol/L.

Abbreviations: M: Males, F: Females, HOMA-IR: Homeostatic model assessment of insulin resistance, CRP: C-Reactive Protein, BP: Blood Pressure, HDL-C: High-Density Lipoprotein Cholesterol.

†Significant sex difference in the prevalence of clinically elevated risk factors (p<0.05).
*Significant sex difference in the prevalence of metabolic syndrome risk factors (p<0.05).

Table 2: Descriptive characteristics of MHO and MUO adolescents by sex and metabolic health status.

	Males (*n*=316)				Females (*n*=316)			
	Primary Definition		Secondary Definition		Primary Definition		Secondary Definition	
	MHO (*n*=142)	MUO (*n*=174)	MHO (*n*=21)	MUO (*n*=295)	MHO (*n*=214)	MUO (*n*=102)	MHO (*n*=27)	MUO (*n*=289)
Age (y)	14.9±0.2	15.5±0.2*	15.3±0.2	15.2±0.1	15.0±0.3	15.0±0.3	14.9±0.4	15.0±0.2
PIR	2.3±0.2	2.6±0.1*	3.1±0.3	2.4±0.1**	2.4±0.1	2.3± 0.2	3.1±0.3	2.2±0.1**
Obesity								
BMI Percentile	97.6±0.1	98.3±0.1**	97.4±0.2	98.1±0.1**	97.4±0.1	97.1±0.2	97.1±0.2	97.6±0.1
WC (cm)	100.2±0.9	108.1±1.3**	96.2±1.1	105.4±0.7*	100.7±0.8	104.5±1.6	95.7±0.6	102.5±0.8**
Diet								
Energy (kcal)	2334±83	2138±93	2210±105	2221±66	1674±58	1738±78	1693±213	1690±42
Total fat (g)	87.8±4.1	77.4±3.8*	82.4±4.5	81.7±3.1	63.2±2.8	64.3±3.8	65.7±11.4	63.2±2.1
Saturated fat (g)	29.9±1.3	26.5±1.3*	29.8±1.4	27.8±1.0	21.5±1.2	23.1±1.4	20.8±4.4	22.1±0.8
Monounsaturated fat (g)	32.9±1.6	28.7±1.4*	30.0±1.9	30.5±1.1	23.5±1.1	24.2±1.5	26.0±4.0	23.4±0.8
Polyunsaturated fat (g)	17.4±1.1	16.0±1.1	15.3±0.9	16.7±0.9	13.1±0.6	11.8±0.9	14.1±2.5	12.6±0.5
Carbohydrates (g)	298.7±10.3	286.4±13.0	285.9±14.8	292.0±8.6	223.0±6.9	232.9±12.6	229.8±23.1	225.1±5.4
Protein (g)	90.1±3.0	79.0±3.6**	85.6±4.3	83.5±2.7	57.6±2.3	60.4±2.9	51.3±7.7	59.3±1.6
Fiber (g)	14.1±0.6	12.6±0.5*	11.6±1.0	13.3±0.4*	10.4±0.4	10.6±0.8	10.2±1.9	10.5±0.3
Cholesterol (mg)	299±18	258±15*	283±32	275±14	188±13	204±17	157±39	197±11
Physical Activity								
MPA (min/week)	192±20	189±41	270±86	184±27**	135±16	124±29	164±73	128±14
VPA (min/week)	314±34	244±25	324±55	269±21*	171±24	140±27	55±34	177±19**
Health Measures								
Insulin-resistance	4.5±0.2	6.8±0.7**	-	-	4.4±0.2	6.6±0.5**	-	-
Inflammation (mg/L)	0.3±0.0	0.2±0.0	-	-	0.4±0.0	0.4±0.1	-	-

Data are presented as means±standard errors.

Abbreviations: MHO: Metabolically Healthy Obesity, MUO: Metabolically Unhealthy Obesity, PIR: Poverty Income Ratio (socioeconomic status), WC: Waist Circumference, MPA: Moderate Physical Activity, VPA: Vigorous Physical Activity.

-Variable not included in model.

*PR significant at $p<0.05$.

**PR significant at $p<0.01$.

Table 3. Associations between obesity, diet, physical activity and laboratory measures with risk of prevalent MHO.

	Males			Females		
		Primary Definition	Secondary Definition		Primary Definition	Secondary Definition
	SE	PR of MHO (95% CI)	PR of MHO (95% CI)	SE	PR of MHO (95% CI)	PR of MHO (95% CI)
Obesity						
BMI Percentile	0.1	0.97 (0.96-0.99)**	0.96 (0.94-0.98)**	0.1	0.98 (0.95-1.01)	0.98 (0.95-1.01)
WC (cm)	0.7	0.92 (0.88-0.96)**	0.92 (0.88-0.96)**	0.7	0.98 (0.96-1.00)	0.95 (0.93-0.98)**
Diet						
Energy (kcal)	63	1.02 (1.00-1.04)	1.00 (0.98-1.01)	46	0.99 (0.98-1.01)	1.00 (0.96-1.03)
Total fat (g)[a]	2.9	1.02 (0.99-1.05)	1.01 (0.97-1.10)	2.3	1.02 (0.97-1.07)	1.03 (0.97-1.09)
Saturated fat (g)[a]	1.0	1.02 (0.99-1.04)	1.04 (1.01-1.08)**	0.9	0.98 (0.93-1.03)	0.97 (0.90-1.04)
Monounsaturated fat (g)[a]	1.1	1.02 (0.99-1.06)	1.00 (0.96-1.04)	0.9	1.00 (0.96-1.05)	1.05 (1.01-1.10)*
Polyunsaturated fat (g)[a]	0.8	1.00 (0.97-1.02)	0.99 (0.97-1.00)	0.5	1.05 (1.01-1.08)**	1.04 (1.00-1.08)
Carbohydrates (g)[b]	8.2	0.96 (0.92-1.00)*	0.99 (0.95-1.02)	5.5	1.00 (0.95-1.04)	1.01 (0.96-1.07)
Protein (g)[a]	2.5	1.03 (1.00-1.05)*	1.01 (0.97-1.05)	1.8	0.99 (0.96-1.03)	0.94 (0.86-1.02)
Fiber (g)[a]	0.4	1.00 (0.99-1.04)	0.98 (0.95-1.01)	0.4	1.00 (0.97-1.03)	0.99 (0.95-1.03)
Cholesterol (mg)[a]	13.0	1.01 (1.00-1.03)*	1.01 (0.98-1.04)	11.0	0.99 (0.97-1.02)	0.97 (0.91-1.03)
Physical Activity						
MPA (min/week)	27	1.00 (0.99-1.02)	1.01 (0.98-1.04)	14	1.00 (0.98-1.03)	1.01 (0.97-1.06)
VPA (min/week)	19	1.01 (1.00-1.02)	1.01 (0.99-1.02)	18	1.01 (0.99-1.03)	0.93 (0.83-1.04)
MVPA (min/week)	35	1.01 (1.00-1.02)	1.01 (0.99-1.03)	22	1.01 (0.99-1.02)	0.98 (0.94-1.03)
Laboratory Measures						
Insulin-resistance	0.4	0.91 (0.88-0.95)**	-	0.2	0.95 (0.92-0.97)**	-
Inflammation (mg/L)	0.0	1.00 (0.99-1.02)	-	0.0	0.99 (0.97-1.01)	-

PR are presented per unit SE and are adjusted for age and PIR (socioeconomic status).
Abbreviations: MHO: Metabolically Healthy Obesity, PR: Prevalent Risk, CI: Confidence Intervals, SE: Standard Error, WC: Waist Circumference, MPA: Moderate Physical Activity, VPA: Vigorous Physical Activity, MVPA: Moderate-Vigorous Physical Activity.
-Variable not included in model.
[a]Further adjusted for energy intake.
[b]Further adjusted for energy intake and fiber.
*PR significant at $p<0.05$.
**PR significant at $p<0.01$.

Table 4. Independent associations between obesity, diet, physical activity and laboratory measures with risk of prevalent MHO.

	Males			Females		
		Primary Definition	Secondary Definition		Primary Definition	Secondary Definition
	SE	PR of MHO (95% CI)	PR of MHO (95% CI)	SE	PR of MHO (95% CI)	PR of MHO (95% CI)
Obesity						
WC (cm)	0.7	0.97 (0.95-0.99)**	0.92 (0.88-0.96)**	0.7	0.99 (0.97-1.01)	0.95 (0.93-0.97)**
Diet						
Saturated fat (g)[a]	1.0	-	1.01 (0.98-1.03)	-	-	-
Monounsaturated fat (g)[a]	-	-	-	0.9	-	1.01 (0.97-1.05)
Polyunsaturated fat (g)[a]	-	-	-	0.5	1.02 (1.00-1.04)*	-
Carbohydrates (g)[b]	8.2	1.01 (0.99-1.03)	-	-	-8.2	-
Physical Activity						
VPA (min/week)	19	1.00 (0.99-1.02)	-	18	-	0.92 (0.82-1.03)
MVPA (min/week)	35	-	1.01 (0.99-1.02)	22	1.01 (0.99-1.02)	-
Laboratory measures						
Insulin-resistance	0.4	0.94 (0.91-0.97)**	-	0.2	0.95 (0.92-0.98)**	

Each column includes the most the most significant obesity, diet, physical activity and laboratory factors for that definition from **Table 3.**
PR are presented per unit SE and are mutually adjusted for the other obesity, diet, physical activity and laboratory variables in the model and are further adjusted for age and PIR (socioeconomic status).
- Variable not included in model.
[a]Further adjusted for energy intake.
[b]Further adjusted for energy intake and fiber.

Abbreviations: MHO: Metabolically Healthy Obesity, PR: Prevalent Risk, CI: Confidence Intervals, SE: Standard Error, WC: Waist Circumference, VPA: Vigorous Physical Activity, MVPA: Moderate-Vigorous Physical Activity.

*PR significant at $p<0.05$.
**PR significant at $p<0.01$.

Chapter Eight: References

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